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THE OPSONO-CYTOPHAGIC TEST IN BRUCELLOSIS*

By ALICE C. EVANS

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During the past year the National Institute of Health sent three investigators into the field to study the prevalence of chronic brucellosis. One of the tests carried out on suspected cases was the opsono-cytophagic test. Altogether slides from about 1,060 cases have been examined at the central laboratory.

As compared with Neufeld's bacteriotropin test, or Wright's opsonic test, the technique of the opsono-cytophagic test is less exacting because the leucocytes remain suspended in the sample of blood tested. It proved to be practical in the field, for all three workers obtained consistent results.

The significance of the opsono-cytophagic test for the diagnosis of brucellosis is another consideration dependent on the percentage of positive reactions found in healthy people. That phase of the problem will be discussed elsewhere. In this paper the method used in carrying out the opsono-cytophagic test is described. It follows the technique described by Huddleson, with certain modifications which were found to improve his technique.

* *Read before the American Society of Medical Technologists, at Atlantic City, N. J., June 9, 1937.*

Technique of the Opsono-cytophagic Test

The blood specimens are collected in 5 cc. amounts in tubes containing 0.2 cc. of a twenty per cent solution of sodium citrate in physiological saline solution. The test is carried out within six hours after collection. The specimens are shaken thoroughly before mixing with the bacterial suspension.

The bacterial suspension is freshly prepared from forty-eight hour glucose agar cultures of *Brucella melitensis* variety *abortus*, No. 456. The growth is taken up in sterile physiological saline solution of pH 7.0, and adjusted to a density equivalent to 600 p.p.m. of the silica standard.

The test is carried out in agglutination tubes. To 0.1 cc. of the citrated blood is added 0.1 cc. of bacterial suspension. After thorough mixing, the tubes are placed in a water bath at 37° C for thirty minutes. Directly after removing the tubes from the water bath, a small amount of the sedimented cells is removed by means of a finely drawn capillary pipette. A large drop of the cell suspension is placed near one end of a thoroughly cleaned and polished glass slide and a spread is made in the usual way by dragging the drop over the slide by means of another slide held at an angle, stopping about a half inch from the end of the slide. Then the top slide is dragged bask, this time being held loosely so that a very thick smear is obtained. The best smears are obtained if the movement is "nervous" giving a wavy, uneven spread.

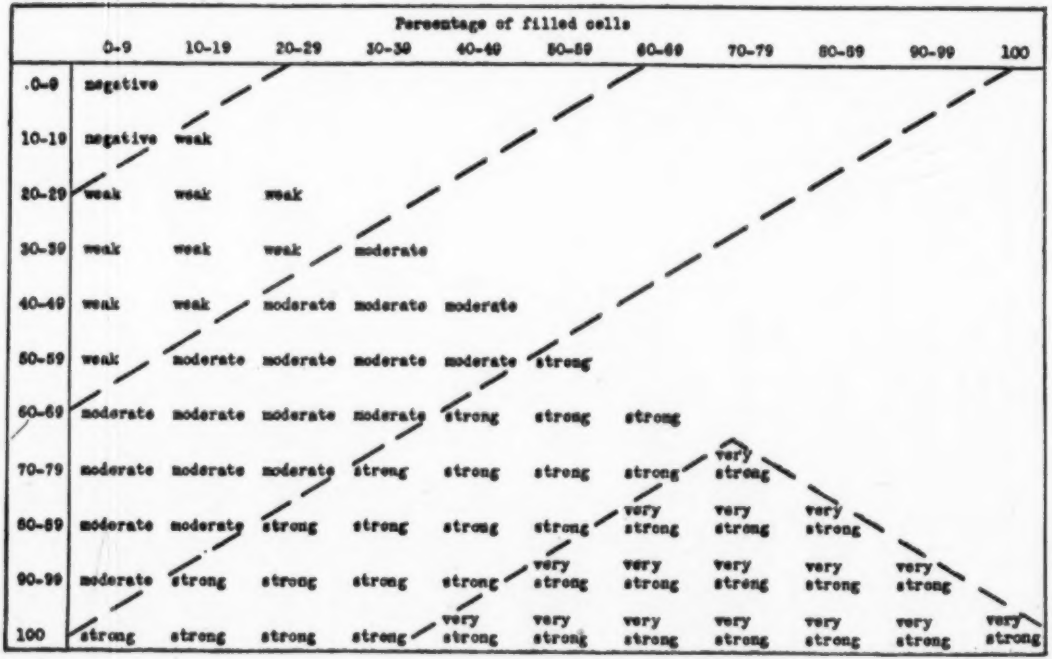
The slides are dried as quickly as possible under an electric fan, in front of an electric heater. They are then treated to dissolve the red cells. The slides are immersed for three or four minutes in a solution containing one per cent acetic acid and five per cent formalin in distilled water. They are then rinsed off, blotted gently on bibulous paper and stained.

The slides are stained with Bordet-Gengou's carbol toluidin blue.[1] Slides stained for about fifteen seconds show the deeply stained nuclei of the leucocytes and the deeply stained bacteria on a clear background.

Discussion of the Modifications

Huddleson's method of carrying out the opsono-cytophagic test was modified in the turbidity standard; in the preparation of the smears; in the staining of the slides; in making the readings and in interpreting the results. A discussion of all these modifications follows.

Chart 1. The interpretation of opsono-cytophagic reactions


Percentage of filled cells
0-9
10-19
20-29
30-39
40-49
50-59
60-69
70-79
80-89
90-99
100
Percentage of positive cells
.0-9 negative
10-19 negative weak
20-29 weak weak weak
30-39 weak weak weak moderate
40-49 weak weak moderate moderate moderate
50-59 weak moderate moderate moderate moderate strong
60-69 moderate moderate moderate moderate strong strong strong
70-79 moderate moderate moderate strong strong strong strong very strong
80-89 moderate moderate strong strong strong strong very strong very strong very strong
90-99 moderate strong strong strong strong very strong very strong very strong very strong very strong
100 strong strong strong strong very strong very strong very strong very strong very strong very strong very strong

Turbidity standard. The silica turbidity standard, which was first adopted by the United States Geological Survey, and was later adopted by the American Public Health Association, is to be recommended for its simplicity, accuracy, and the readiness with which it may be prepared in any laboratory. Its preparation is described in every edition of *Standard Methods of Water Analysis.* In our laboratory a single standard preparation is used—a suspension of 300 parts per million of silica in an 8 cc. homeopathic vial sealed with a paraffin covered cork stopper. Thus prepared, it may be kept indefinitely without deterioration.

The choice of standard with a density of 300 parts per million of silica in an 8 cc. homeopathic vial was made because at this density black letters of ordinary type are barely legible through it. At this degree of legibility comparisons may be made with considerable accuracy.

The one standard serves to prepare a suspension of any desired density. For example, to prepare a bacterial suspension of a density equivalent to 600 p.p.m. of the silica standard as used for the opsono-cytophagic test, the procedure is as follows:

Into an 8 cc. homeopathic vial 0.2 cc. of the dense bacterial suspension is placed, and water is added until the density matches that of the standard. If it requires 3.8 cc. of water to bring 0.2 cc. of the heavy bacterial suspension to the density of the 300 p.p.m. standard, then 3.6 cc. of saline solution should be added to 0.4 cc. of the heavy bacterial suspension to bring it to a density equivalent to 600 p.p.m. of the silica standard. A *Brucella* suspension of this density contains approximately five billion organisms per cubic centimeter.

Preparation of the smear. At first the slides which we received from the field often had smears so thin that it took a long time to find enough leucocytes to make a satisfactory reading. In response to a request that the smears be made thicker, the described technique for preparing the slides was devised by Dr. Royall M. Calder, who conducted the investigation in San Antonio, Texas.

Staining the slides. The Hasting's stain which is recommended by Huddleson was found to be less satisfactory than

[1] Bordet-Gengou's carbol toluidin blue is made by dissolving 5 grams of toluidin blue in 100 c.c. alcohol, 500 c.c. distilled water, and 500 c.c. of five per cent phenol. One part of this dye is diluted with two parts of distilled water for staining the smears.

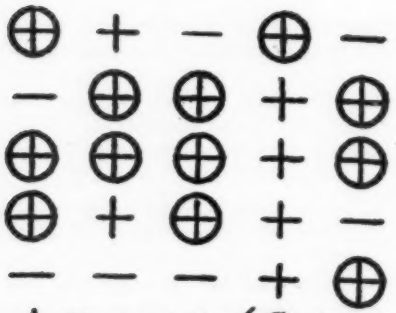
Bordet-Gengou's carbol toluidin blue which we had found most satisfactory in phagocytic studies made many years ago.

Reading the slides and interpreting results. For the reading of slides and the interpretation of results we have devised a method more simple than Huddleson's.

The wearisome task of counting bacteria ingested by leucocytes was omitted without loss of accuracy in interpretation. Indeed, the counting of ingested bacteria may be a source of error when the numbers are few, because it is not the numbers of bacteria that appear to be within the leucocytes which determines whether the reaction of the given cell is positive or negative. The significant point to be determined is whether the bacteria which appear to be within the leucocytes are more numerous than in an equivalent area of the surrounding field.

If two samples of blood are tested with bacterial suspensions of exactly the same density, and smears are prepared of exactly the same thickness, the number of bacteria in a field the size of a leucocyte may differ greatly on the two slides depending on whether or not agglutination has taken place. Negative chemotaxis does not occur. Hence, on one slide there may be an even distribution of bacteria, with some lying over the leucocytes and appearing to be ingested. On the other slide the bacteria may be agglutinated with most of the inactive leucocytes standing in a clear field. Thus, if the presence of bacteria apparently within a leucocyte is regarded as signifying a positive reaction, many of the cells of the first slide may be erroneously recorded as positive, whereas that error would not be so likely to be made on the second slide. For this reason a rapid decision, made at a glance which takes in the surrounding field as well as the leucocyte, may result in more accurate judgment than the arduous counting of cells.

Twenty-five polymorphonuclear leucocytes on different parts of the slide are examined. Leucocytes with no more bacteria than in a corresponding area of the surrounding field are recorded as negative. Those with more bacteria than are in the surrounding field are recorded as positive; and if the leucocyte is well filled with bacteria a circle is drawn around the plus sign. A leucocyte is regarded as "filled" if it contains approximately forty bacteria or more. The following reading of a slide offers an illustration. Percentages are obtained by multiplying the number of negative, positive or filled cells by four:



— 28 per cent; + 72 per cent; (⊕ 48 per cent)

The interpretation of the reading is made according to the accompanying chart.

According to this system of interpretation, the total percentage of reacting leucocytes receives a rating on the vertical scale, and the rating is increased on the horizontal scale, according to the percentage of filled cells. There are five degrees in the rating of a reaction-negative, weak, moderate, strong, and very strong. Thus, a reading of seventy-two per cent positive cells with twenty-four per cent of them filled would be interpreted as a moderately positive reaction; with forty-eight per cent of cells filled the reaction would be interpreted as strong; with all of the reacting leucocytes filled the reaction would be interpreted as very strong.

In a general way, this system of readings may be compared with Huddleson's as follows: We would record with a plus sign leucocytes which he would record as slightly or moderately positive. We would record with an encircled plus sign the leucocytes which he would record as markedly positive.

In the consideration of only twenty-five leucocytes on a slide we follow Huddleson's practice. We believe that other inaccuracies inherent in the technique would obviate further refinement of the readings by the examination of a larger number of cells.

In spite of the various sources of error with which the investigator must contend in preparing and reading the slides, the final result leading to an interpretation of negative, weak, moderate, strong or very strong phagocytosis may be obtained in repeated tests with considerable accuracy.

When the test is carried out with normal serum a few leucocytes often appear to have engulfed bacteria. Hence, it would be erroneous to interpret such a test as positive. The line of division between a reading regarded as negative or weakly positive as given on the chart is necessarily arbitrary, as is the line of division between the higher degrees of phagocytosis.

The writer tested the accuracy of her own interpretations by rereading one hundred slides received from field investigators. The slides to be reread were first chosen from the records of the previous readings. They were taken in consecutive order, excepting that several series of slides with negative readings were omitted, so that a goodly percentage of positive readings might be included in the one hundred slides.

Readings were then made without knowing the previous rating. On eighty-two per cent of the slides the second interpretation agreed with the first. On fifteen per cent of the slides there was one degree of error, as, for example, a slide was interpreted as weakly positive in one reading and moderately positive in the other. A third reading confirmed either the one or the other previous reading. On three per cent of the slides, all of which were poorly stained, there was greater error.

From the above results it may be concluded that well stained slides may be read with considerable accuracy; and that it is useless to attempt to make readings on poorly stained slides.

BIBLIOGRAPHY

Francis, Edward. Culture medium for Bacterium *tularense*. (This leaflet may be obtained from the National Institute of Health, Washington, D. C.)

Huddleson, I. Forest. 1934. Brucella Infections in Animals and Man. The Commonwealth Fund, New York.

BILE CHEMISTRY

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In order to understand the significance of "Bile Chemistry," it is well to examine the problems which prompted its development. The liver has many different and independent functions: (1) The one in which we are most interested, namely, it manufactures and excretes bile pigment, cholesterol and bile salts.

(2) It stores carbohydrates as glycogen and helps maintain the blood sugar level.

(3) It detoxifies many substances, the most important of which are the protein cleavage products which come to it in the portal circulation from the intestines during digestion.

(4) It produces fibrinogen.

(5) It removes foreign substances and bacteria from the blood stream. With so many distinct functions, it is impossible to devise a single test which can give an accurate picture of the condition of the liver; in fact, methods have been developed to test each function in turn.

Interference with the excretion of bile pigment or bilirubin is probably the most obvious sign that all is not well with the liver. Until the early part of the present century, the only measure of jaundice was the tint of the skin, eyes and mucus membranes, and the presence of bile in the urine and feces. In 1913, van den Bergh published his momentous experiments on the detection of bilirubin in blood serum. Bilirubin is manufactured from hemaglobin by the reticulo-endothelial cells all over the body, but most of it is made in the spleen. This has been proven by the fact that blood leaving the spleen contains more bilirubin than blood entering it. In the liver this material is extracted from the blood by the gland cells lining the bile capillaries which are closely twined about among the capillaries connecting the portal and hepatic veins, and conduct the bile into the larger bile ducts which eventually empty into the gall bladder. If these ducts become obstructed in any way, this bile is again absorbed into the blood, but differs somewhat from the bilirubin which has not passed through the liver cells. Thus, jaundice may arise from two causes: obstruction of the bile ducts or dam-

age to the liver cells which prevents them from extracting the bilirubin manufactured in the spleen and elsewhere.

In 1860, a German chemist named Greiss discovered what is known as the diazo reaction, a process of fundamental importance in organic chemistry. Upon it depends the whole science of manufacture of artificial dyes. The principle involved is that, when aniline or some other aromatic amine is dissolved in hydrochloric acid and treated with sodium nitrite, two molecules of water are eliminated and the hydrochloride of a strong base is formed. The diazonium group may be compared to the ammonium group—it does not exist alone but forms an hydroxide and a series of stable and crystaline salts. Two theories have been advanced to explain the structure of the diazonium molecule. Kekule thought that there was a double bond between the two nitrogen atoms, C_6H_5N: NCl, but the formula proposed by Blomstrand, C_6H_5NCl:N, is the one more generally adopted. Besides the dyes for our clothing, many of the stains and indicators used in our laboratories are examples of these diazo dyes: methyl orange, congo red, bismark brown, Tofler's reagent are some of them.

In 1883, Ehrlich discovered that if a small amount of a diazonium salt in acid solution be added to an alcoholic solution of bilirubin, coupling takes place with the formation of a red azo-dye, azo-bilirubin. This was isolated by Proscher in 1900, but was not made use of in clinical work and was forgotten till van den Bergh resurrected it. He found that the reaction is sensitive to one part of pure bilirubin dissolved in 1,500,000 parts alcohol. He also pointed out that there are two types of reaction. In one, the coupling takes place directly in icteric sera; in the second, coupling takes place only in alcoholic solution. Ethyl alcohol, methyl alcohol or acetone may be used. He explained these types on the basis that the first, or direct, reaction occurs only with bile from the gall bladder or bile which has passed through the liver cells. This happens in what he classifies as mechanical jaundice due to obstruction. This kind of bile is also more easily oxidized to biliverdin. The second type, or indirect reaction, occurs with bilirubin obtained from blood serum or serous fluid from chest or abdomen, pigment which has not passed through the liver cells. This happens in what he terms dynamic jaundice and includes by far the greater number of cases: hemolytic jaundice and the great variety of toxic and infectious jaundice which may be caused by certain fevers, the administration of some drugs, damage to the liver cells by cirrhosis and atrophy, and such conditions as acute sepsis and cardiac disease with back pressure. In this type, bile is absent from the urine but present in the feces.

The method devised by van den Bergh is relatively simple. The diazo reagent is prepared from sulphanilic acid and sodium nitrate. The two solutions are fairly stable but must be mixed immediately before using.

Solution No. 1:	sulphanilic acid	1 gm
	hydrochloric acid (conc)	15 cc
	water qs	1000 cc
Solution No. 2:	sodium nitrite	.5 gm
	water qs	100 cc

Use 25 cc of No. 1 and add .75 cc of No. 2.

For the Direct Reaction, set up three small tubes, placing .25 cc serum in each. To the first add .2 cc water, to the second .2 cc Diazo Reagent, and observe for one minute. Then add .2 cc Diazo Reagent to the third tube to act as a color comparison. If the color change, red to violet, begins immediately and is complete in one-half to two minutes, the reaction is positive direct and indicates obstructive jaundice. If the color change begins after 30 seconds and deepens slowly and gradually, the reaction is a delayed direct and is classed as an indirect. If there is no color change, there still may be a positive indirect which takes place only in alcoholic solution.

For the Indirect Reaction, place 1 cc clear serum in a graduated centrifuge tube, add .5 cc Diazo Reagent and let stand for one to two minutes to allow coupling to take place. This is especially important when the direct reaction is positive, as bilirubin is less soluble than azobilirubin and may be carried down with the precipitated protein. Then add 2.5 cc 95% alcohol and 1 cc saturated solution of ammonium sulphate; this precipitates the albumen and globulin. For some colorimeters, it is necessary to double these quantities. Mix well and centrifuge. The contents of the tube will arrange themselves in three layers: first the ammonium sulphate solution, next a layer of precipitated proteins, and above this the beautiful, clear red alcoholic solution of azobilirubin. The dilution is now determined by the amount of this solution as all the bilirubin in 1 cc of serum is contained in it—usually about 3 cc. This is pipetted into a colorimeter cup to be compared with a standard.

Because of the difficulty in obtaining pure bilirubin, two very satisfactory artificial standards have been devised. One consists of a dilute solution ammonium iron alum, to which is added, immediately before using, an equal amount of 20% solution potassium thiocynanate and 12 cc of ether. The red iron salt is more soluble in ether than in water and appears in the ether layer after thorough shaking. This is carefully pipetted into the "standard" colorimeter

cup and equals 5 mg bilirubin per liter, or 1 van den Bergh Unit.

The other standard is a permanent one; it contains 2.11 gm anhydrous cobaltous sulphate in 100 cc water to which .5 cc sulphuric acid has been added. Since cobaltous sulphate decomposes slightly when heated to drive off the water of crystalization, and the composition of the crystaline salt is not constant, it is recommended that the solution be made up too concentrated and diluted to match the iron standard. This standard keeps well, especially in the dark. The amount of bilirubin in the serum is calculated by this formula:

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times \text{dilution} \times 5 = \text{mg bilirubin per liter}$$

Slight hemolysis does not interfere with the test, but the presence of chyle does, so it is best to draw the blood when the patient is fasting. The normal range is one to three mg per liter, or .2 to .6 van den Bergh unit. In cases of complete obstruction the values may reach 50 units or 250 mg. Pernicious anemia sometimes gives as high as 5 or 6 units—always of the delayed type. The values found in gall bladder drainage vary greatly, sometimes reaching 50 to 60 units direct.

Another very simple test is the Icteric Index. This was devised by Meulengracht and improved by Bernheim. It consists of a color comparison of serum with an arbitrary standard, 1:10,000 solution potassium bichromate, .050 mg in 500 cc water. Icteric sera must be diluted with .9% sodium chloride solution till the color is nearly equal and the match made in a colorimeter. Water may be used to dilute the serum if a drop or two of 10% hydrochloric acid is used to dispel the cloudiness. Other pigments such as carotin and even slight hemolysis interfere with this test, especially if the values are low. Therefore, use either a dry syringe or one rinsed with saline to draw the blood. Normal range is from 4 to 6, latent jaundice from 7 to 16, and clinical jaundice above 16. Perhaps these techniques do not coincide with those in your favorite reference book. I found an amazing variation. These are taken from a U. S. Naval Bulletin of which Hall is the author, and are the ones I use.

In order to test the second liver function, its ability to store carbohydrates, a number of tolerance tests have been devised, none of which meets with unqualified approval. The glycogen content of even normal liver varies greatly, from a minimum of 1% to 20%. Although the liver can manufacture glycogen from levulose, the muscles can also do this and utilize it independently of the liver. For this reason, levulose is a poor measure of hepatic function.

Galactose utilization depends largely upon the liver but varies widely even in normal animals, so it also is open to criticism.

In order to test the third function, its relation to protein metabolism, several tests have been invented. In jaundice, the total serum protein is usually somewhat reduced and the albumen-globulin ratio is altered, sometimes to the extent of being reversed. A test which appears to depend upon this alteration is the Takata reaction. There has been very little mention of this phenomenon in American literature and the claims for its value differ greatly. Snell of the Mayo Clinic considers it very optimistically as a differentiation between cirrhosis and metastatic lesions, conditions which simulate each other closely, but other writers do not seem to share his views. The test is very simple: Into each of six small tubes, put 1 cc .9% saline. To the first tube add 1 cc serum; mix well and transfer 1 cc to the second tube and so on, discarding the last cc; this gives dilutions of 1:2 to 1:64. Now add .25 cc 10% solution sodium carbonate to each tube, followed by .15 cc .5% mercuric chloride sol. Shake well and read in 5 minutes and in 24 hours. A pearly flocculent precipitate filling at least one fourth of any tube and a definite precipitate in at least four tubes is considered strongly positive. All other types of precipitate or lesser degrees are considered negative. Heath in the New England Journal of Medicine reports positive readings in 60% of his cases of cirrhosis and positives in less than 3% of general hospital cases showing no liver damage. Kirk in the Oct., 1936, J. A. M. A., reports 84% positive readings in his cases of cirrhosis and 3% in general hospital cases. Nevertheless, Kirk concludes that the test is not diagnostic of cirrhosis but is positive in any case of increased globulin content.

Another test of this third function is the Widal Crisis or Hemoclastic Crisis. A leucopenia is produced following ingestion of protein in cases of liver impairment. Hall thinks the value of this test questionable.

The ability of the liver to manufacture fibrinogen may be tested by merely determining the fibrinogen in the plasma. It is markedly decreased in certain forms of jaundice, which accounts for the fact that icteric blood clots slowly.

And lastly, its ability to remove foreign substances may be tested by determining the rate at which dyes administered intravenously disappear from the blood. The most successful of these methods is the Bromsulpthalein Test devised by Rosenthal. 2 mg. of dye per kilo of body weight is given and blood withdrawn from the opposite arm in 5 minutes and again in 30 minutes. In normal

cases, only 20% to 50% of the dye remains in 5 minutes, and only a trace or none at all at the end of 30 minutes. Standards may be prepared by dissolving 4 mg dye in 100 cc water made alkaline with .25 cc sodium hydroxide. This gives the 100% standard and nine others may be made from this by suitable dilutions. If they are sealed in tubes and kept in the dark the colors are reasonably permanent. To determine the amount of dye still remaining, the blood is centrifuged and the serum pipetted into two tubes of the same diameter as those containing the standards. One is made alkaline with one or two drops of sodium hydroxide and placed between two standards; the second tube is held in front of the standard to act as a light filter. Since hemolysis and the presence of chyle interfere with this test, great care must be taken that none occurs. This test measures the extent of liver damage where such damage is diffuse as in cirrhosis but gives normal values if only a small portion of normal liver tissue remains. Hall in a series of cases of cholangitis, found that the van den Bergh, Icteric Index and Bromsulphalein Liver Tests paralleled each other very closely, the latter being the first to return to normal.

BIBLIOGRAPHY

Hall, U. S. Naval Med. Bul. xxiv, 4. 1924.
J. M. McNee, Quar. Jour. Med. XVI, July, 1923.
F. C. Mann, Annals of Int. Med., VIII, 1934.
A. M. Snell, Collected Papers of Mayo Clinic XXVII, 1935.
Robt. Kirk, J. AMA., 107, Oct., 1936.
J. B. Cohen, Theoretic Organic Analysis.
Heath, N. Eng. M. J., 211, Dec., 1934.

A SIMPLE SLIDE TEST FOR SYPHILIS*

By SEABORN J. LEWIS

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The Wassermann reaction and several of the precipitation methods for syphilis have reached a very high degree of accuracy. I have, therefore, long hesitated to publish my results with a modification of the precipitation reaction. Having performed serological tests with some 27,000 serums, I feel it desirable to present the technic. Over 11,000 serums were tested by the Noguchi[8] method, 2,400 by the Wassermann (a modified Kolmer),[5] approximately 10,000 by the Kolmer, Kahn,[4] and "Lewis," 1,500 by the Kolmer, Kahn, Kline,[6] and "Lewis," and about 1,500 by the "Lewis" alone. These 1,500 serums were from prospective transfusion donors where rapid results were necessary. Many of these serums were run several times with several trial and error methods. In addition, a great many hundreds of serums were run with Butler at Tulane University, using various Wassermann and precipitation methods. In 1926, Butler[1] published his slide precipitation test for syphilis, and in 1929 another article of his[2] appeared, based on the Meinicke.[7] Later Johns,[3] published a modification of the slide test method, and Venable[9] also reported on such tests.

The chief difficulty in perfecting a satisfactory rapid slide test for syphilis has been in producing a uniform and stable antigen. By manipulation of the antigen constituents, an antigen can be had that is of a standard, low, or high degree of sensitivity and specificity. But constant duplication of an antigen of the proper degree of sensitivity has been a problem. The following procedures and technic were found to be satisfactory, and to give reliable results:

METHOD

Stock Antigen Preparation

100 cc. of beef heart extract prepared exactly according to Kahn.

1.5 grams of powdered balsam of tolu.

0.4 gram of cholesterol (Pfanstiehl).

After adding the tolu to stoppered container shake vigorously for a few minutes and place in the incubator. Repeat the shaking twice a day for two days. Add the cholesterol and shake for a few minutes, place in the incubator for two days, and shake again twice

* *Reprinted by permission of American Journal of Clinical Pathology, Vol. 6, No. 5, September, 1936.*

a day. Filter and store in a glass stoppered bottle, or in one with a tin foil covered cork. The stock antigen, kept in the incubator or at room temperature in warm climates, remains stable for months. Protect it from the light.

The balsam of tolu is dried in the incubator until it is crisp or brittle, then placed between several thicknesses of brown wrapping paper, and hammered until pulverized. The coarse particles are discarded by using a hand sifter.

Saline Benzoic Acid Diluent

100 cc. distilled water.

3 grams sodium chloride, c.p. (Baker's).

2 cc. of a one per cent alcoholic solution of benzoic acid.

Technic for Performing the Test

Preparation of antigen mixture: Into a small test tube measure 0.1 cc. of stock antigen and add 0.3 cc. of the saline diluent. At once shake the tube for a moment, and mix thoroughly for a few seconds by means of a capillary pipet with a rubber bulb, or a medicine dropper. Allow the mixture to stand for five minutes, and agitate again just before using. Use the antigen mixture within thirty minutes of its preparation. Some lots should be used almost immediately after mixing.

Two drops of clear serum that have been heated at 56° C. for thirty minutes, are placed on a slide, and adjacent to the serum three drops (an equal quantity) of the antigen mixture. Mix with the broader flat end of a toothpick so that the mixture occupies the middle two thirds of the slide, and is not near enough to the sides to run over. The stirring should result in an oval spread with smooth margins so as to facilitate a rotary and side to side motion, and to prevent running over. Rock the slide from side to side for three minutes, and read within five minutes. Read by indirect light against a dark background, or by holding in front of, and just above a daylight bulb. A board painted black and with thin partitions to hold ten slides is convenient for running a number of serums. Ten is about the maximum number that can be properly mixed and agitated at one time. The test should not be performed in a cold room, as this causes a tendency towards false positives. Fans and strong drafts should be avoided.

Interpretation: Negative serums have a smooth, cloudy, opalescent appearance. Positive serums show various degrees of a brownish granular precipitate, easily seen. Very strongly positive

serums will show large clumps floating in an almost clear fluid. A minute amount or degree of precipitation should be disregarded or checked by other methods.

Experimental Notes

Many combinations and variations of the constituents of the antigen described have been tried. The following conclusions are drawn:

(a) Two parts of diluent to one part of stock antigen, or four parts of diluent to one part of stock antigen give about the same results as the three to one proportion advocated above.

(b) Substituting normal saline and benzoic acid gave poor results.

(c) 3 per cent saline without benzoic acid, using heated serum, gives about equal results as with acid.

(d) 3 per cent saline without benzoic acid, using unheated serum, gives about equal results as with acid.

(e) Heated serum with benzoic acid gives clearer cut, more sensitive and specific results than any other combination.

(f) The factor of the pH is ignored in this technic, though sensitivity may be increased by adding hydrochloric acid, or decreased by adding sodium hydroxide.

(g) The presence of citrate in serum was found to be undesirable, rendering positive serums almost negative in reaction. This is important, as often one has at hand only citrated blood obtained for hematologic, chemical, or cultural purposes.

(h) Kahn and Kolmer antigens used according to the author's technic were unsatisfactory.

(i) Kahn and Kolmer antigens used according to Kline's technic were unsatisfactory.

(j) The author's antigen mixture may be substituted for the Kline antigen mixture and used as in his technic with good results.

(k) 1.25 per cent to 1.5 per cent balsam of tolu appeared to be the optimum amounts. Less tolu leads to too sensitive results, while greater amounts tend to obscure the reaction.

(l) Cold temperatures increase the antigen sensitivity, and warm temperatures decrease it.

Stock Antigen Adjustment

The above formula usually gives reliable results, but this antigen, as is true of all antigens, may require adjustment. Every new lot of antigen should be tested with known negative and all degrees of known positive serums from individuals with treated and untreated syphilis in all stages.

If the above formula results in antigen that is too sensitive, desensitizing reagent should be used. This is beef heart extract containing 1.5 per cent balsam of tolu. One part of desensitizing reagent should be added to nine parts of the new stock antigen. This mixture must be tried out with known negative and positive serums, and if necessary smaller or greater proportions of densitizing reagent should be added to the new antigen until the latter is of standard sensitivity. The entire new lot can then be corrected.

Antigen, less sensitive than standard can be corrected in like manner by adding sensitizing reagent to the new lot of antigen. The sensitizing reagent consists of beef heart extract containing 0.55 per cent cholesterol and 1.5 per cent balsam of tolu.

Discussion

The antigen prepared and used as above has given results equal to the Wassermann, or any of the standard precipitation procedures. No attempt is made at this time to give figures or comparative results. In no instance has this test been strongly positive when the Kolmer, Kahn, and Kline were negative. In treated syphilis all of the standard precipitation technics and mine have proved somewhat more sensitive than the Wassermann. Extremely rarely has this technic given a negative or a moderately positive result that did not agree with the majority of three standard methods. The test has been used primarily to rule out syphilis, and where occasionally a weakly positive result has been obtained with this test, the result has been disregarded as evidence of syphilis unless the standard methods checked.

Most diagnostic laboratories employ, as I do, several methods in the serological diagnosis of syphilis. But the technic described is useful in ruling out the presence of syphilis, or in conjunction with one or more other methods.

Summary

A simple, reliable slide test for syphilis is described. The results with thousands of serums have compared most favorably with several of the recognized methods used in the diagnosis of syphilis. The test is a modification of, and is based upon, the work of others who have utilized the precipitation principle in the serology of syphilis.

BIBLIOGRAPHY

1. Butler, H. W.: A simple, rapid precipitin test for the diagnosis of syphilis. New Orleans Med. and Surg. Jour., 79: 105-106. 1926.
2. Butler, H. W.: The adaptation of standard antigens to the precipitin test for syphilis, with comparative results shown by three thousand sera. Jour. Lab. and Clin. Med., 14: 336-341. 1929.
3. Johns, F. M.: Another modification of the precipitin test for syphilis. New Orleans Med. and Surg. Jour., 83: 279-282. 1930.
4. Kahn, R. L.: The Kahn test. Baltimore: Williams & Wilkins Co., 1928, pp. 201.
5. Kolmer, J. A.: Studies in the standardization of the Wassermann reaction. Am. Jour. Syphilis, 6: 1-32. 1922.
6. Kolmer, J. A. and Boerner, Fred: Approved laboratory technic. New York: D. Appleton & Co., 1931, pp. 663.
7. Meinicke, Ernst: Ueber eine neue Methode der serologischen Luesdiagnose. Berl. klin. Wchnschr., 54: 613-614. 1917.
8. Stitt, E. R.: Practical bacteriology, blood work and animal parasitology. Philadelphia: P. Blakiston's Son & Co., 1927, pp. 837.
9. Venable, D. R.: The Butler-Meinicke reaction. Texas State Jour. Med., 28: 409-412. 1932.

NEWS AND ANNOUNCEMENTS

REGISTRY OF MEDICAL TECHNOLOGISTS
OF THE
AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

There were 566 applicants scheduled to appear for the Registry examination in October, of which 468 actually took the written and practical tests. Information as to the successful applicants is not as yet available.

The following are the questions which were used for the written test:

1. Show by diagram the ruled area of the counting chamber used to count white and red cells, and give dimensions.
2. Give the figures for the following:
   (a) Normal erythrocyte count.
   (b) Normal leukocyte count.
   (c) Normal differential count.
   (d) Normal amount of hydrochloric acid in stomach contents.
   (e) Normal number of leukocytes in spinal fluid.
3. Define the following:
   (a) Hypochromic anemia.
   (b) Macrocytic anemia.
   (c) Leukemia.
   (d) Infectious mononucleosis.
   (e) Leukopenia.
4. How would you prepare the following reagents?
   (a) Acid alcohol.
   (b) Physiologic salt solution.
   (c) Normal oxalic acid.
   (d) Wright's stain.
5. List the reagents used in the following tests (do not give technique):
   (a) Blood sugar.
   (b) Gastric analysis (chemical).

(c) Mastic test.
(d) Acetone in urine.
(e) Wassermann test.

6. Give the technique for Friedman's test for pregnancy.

7. Give the technique for preparing a frozen section of tissue for microscopic examination.

8. Give the technique for the following:
   (a) Agglutination test for undulant fever.
   (b) Kahn test for syphilis.

9. A. Give the technique for (1) the typing of blood, (2) cross matching of blood.
   B. Show with drawings: (1) a compatible test, (2) agglutination of cells, (3) Rouleaux formation.

10. Give the reaction to Gram's stain, a suitable culture medium for growth, and one cultural characteristic for the following organisms:
    (a) Diplococcus pneumoniae (pneumococcus).
    (b) Eberthella typhi (Bacillus typhosus).
    (c) Corynebacterium diphtheriae (Bacillus diphtheriae).
    (d) Neisseria intracellularis (meningococcus).
    (e) Escherichia coli (Bacillus coli).

NATIONAL

Members of the American Society of Medical Technologists who may wish to present papers at the annual meeting in San Francisco, or who have suggestions concerning the program please communicate with Bernice Elliott, 5107 Webster St., Omaha, Nebraska, before March 1.

The officers of the American Public Health Association announce that the 67th annual meeting will be held in Kansas City, Mo., October 25-28, 1938.

Dr. Edwin Henry Schorer, Director of the Kansas City Health Department, has been appointed chairman of the local committee. He will be assisted by a large group of city and state officials and community leaders.

A long list of affiliated organizations meet habitually with the American Public Health Association. They include:

The American Association of School Physicians
The Association of Women in Public Health

The Conference of State Laboratory Directors
The Conference of State Sanitary Engineers
The American Association of State Registration Executives
Delta Omega
The International Society of Medical Health Officers
The attendance at the 67th annual meeting will exceed 3,000 professional public health workers from every state in the Union, Canada, Cuba and Mexico.

Pennsylvania

The Pennsylvania Society of Medical Technologists held its fourth annual dinner meeting on October 25 in the Mirror Room of Whitman's, Philadelphia. Cora Louise Miller, the state president, gave the address of welcome and introduced Dr. Fred Boerner of the Graduate School of Medicine of the University of Pennsylvania who served as our toastmaster. A great part of the success of the evening was due to Dr. Boerner. Dr. Russell Richardson, head of the Cox Foundation of the University of Pennsylvania, was the guest speaker of the evening, giving us a timely address on technicians' profession rather than trade. Dr. John A. Kolmer of Temple University talked to us concerning the idea of having a state convention or institute and offered his services in the event we decided to hold one (plans are now under way). Dr. Frank W. Konzelmann, also of Temple University, an honorary member of the Society, gave a short address as did several other physicians present and our officers. The Society was pleased to have as its guest Frieda Ward, its first president and now president of the American Society of Medical Technologists.

South Dakota

A meeting of the South Dakota State Medical Technologists was held at Sioux Falls with a good attendance considering the inclemency of the weather, about twenty technicians were present and several non-members. The meeting was held in the city hall, courtesy of President Harry Falconer, who is with the Public Health Department of that city.

Among the important measures were the following: A committee appointed to make formal application for acceptance in the Inter-Allied Medical Council.

Place for 1938 meeting was voted on in favor of the Black Hills Rapid City, being the selected headquarters. Meeting will consist of a two-day session beginning about September 1st.

Informal discussion following each doctor's address brought out many new points in various laboratory procedures.

New officers elected were: Mr. Samuel Crabb, President; Mr. Harry Falconer, Vice-President; Mrs. Oretta Axtell, Secy.-Treas.; Miss Lois Van Tassell, New Counselor.

The following program was presented:

8:30 to 9:00 a. m.—Registration, Third Floor, City Hall.

9:00 a. m.—Meeting will be called to order.
Address of Welcome, Hon. Mayor A. N. Graff.
Discussions.
Election of Officers.
Reports.

1:30 p. m.—Report of Venereal Clinic and Food Handlers Examination. Dr. Emil Ericksen, City Health Officer.
Report on International Society of Rentnologists meeting held in Chicago, Ill. Dr. N. J. Nessa, Sioux Falls Clinic.
Demonstration of the Alveolar Apparatus. Sister M. Leonard, McKennan Hospital.
The remainder of the program will be given at the Sioux Valley Hospital.

3:30 p. m.—Laboratory Ethics. Miss Agnes B. Thompson, superintendent of Nurses, Sioux Valley Hospital.
Crippled Children Clinic. Dr. Guy E. Vandemark, Sioux Falls Clinic.

6:15 p. m.—Banquet at the Community House. Following the banquet, Miss Rowena Bliss will introduce Mr. Ben Fantle who will give a foreign travelogue.

Tepee Day

8:30 p. m.—Football game at the East Side Athletic Field—Sioux Falls College vs. Western Union College.

All South Dakota Technologists are urged to attend this meeting.

Harry Falconer, M.T., Pres.
Mrs. Bernice Iverson, M.T., Vice-Pres.
Sister M. Hermina, Sec.-Treas.

BOOK REVIEWS

A TEXTBOOK OF HISTOLOGY, SEVENTH EDITION, by Harvey Ernest Jordan, A.M., Ph.D., Professor of Histology and Embryology, University of Virginia. Pp. 738. Published by D. Appleton-Century Company, 35 West 32nd Street, New York.

This work has been a standard textbook of histology since the first edition published in 1916. There are 610 illustrations, thirty-nine having been replaced by new and improved figures. The book is intended primarily for medical students. Histology is the foundation on which medicine is built. Most medical students accept it as a necessary evil and a hard uphill road which has to be traveled in order to reach the more interesting heights of clinical medicine. The author being a teacher, and therefore a student of students, realizes this and so has interspersed his work where feasible with a description of the functions of various tissues in order to stimulate and hold the student's interest in what to him is ordinarily considered a "dry" subject. For this the author is to be commended as well as for his excellent, concise, thorough and accurate presentation of the subject.

BACTERIOLOGY OF SPECIFIC COMMUNICABLE DISEASES. A Handbook of Public Health Bacteriology. General information regarding epidemiology, collection and shipment of specimens, and bacteriologic, serologic and chemical procedures. Edited by M. S. Marshall, Department of Public Health, City and County of San Francisco, California. Publishers, J. W. Stacey, Inc., 236 Flood Building, San Francisco.

ABSTRACTS

PHAGOCYTIC ACTIVITY OF CIRCULATING CELLS IN THE VARIOUS TYPES OF LEUKEMIA: M. M. Strumia and F. Boerner. Amer. Jour. Path., Vol. 13, No. 3, p. 335, May, 1937.

The phagocytic activity of the various types of blood is compared. Lymphocytic cells do not show phagocytosis. The phagocytic activity of granulocytic cells increases with the approach to maturity up to the rod nuclear form. Monocytic cells, eosinophiles and basophiles show less activity than polymorphonuclear neutrophiles.

CULTIVATION OF THE VIRUS OF ST. LOUIS ENCEPHALITIS: R. W. Harrison and E. Moore, Amer. Jour. Path., Vol. 13, No. 3, p. 361, May, 1937.

Report of the cultivation of strains of the virus of St. Louis encephalitis in vitro by using suspensions of minced mouse and chick embryo.

BLASTOMYCOSIS OF THE HEART: R. D. Baker and E. W. Brian, Amer. Jour. of Path., Vol. 13, No. 1, p. 139, Jan., 1937.

Report of two cases of generalized infection with Blastomyces dermatitidis in which autopsy showed blastomycosis of the heart. The authors discuss the similarity of cardiac involvement in blastomycosis and tuberculosis.

ACUTE BACTERIAL ENDOCARDITIS DUE TO PSEUDOMONAS AERUGINOSA (B. PYOCYANEUS): G. W. Fish, M. M. Hand and W. F. Keim, Jr., Amer. Jour. of Path., Vol. 13, No. 1, p. 121, Jan., 1937.

Report of a fatal case of generalized B. pyocyaneus with bacterial endocarditis. The author traces the infection to the hypertrophied prostate gland which, when removed surgically, showed Gram negative bacilli.

A SIMPLE METHOD FOR THE DETERMINATION OF ACETONE IN BLOOD AND URINE: J. C. Abels, Jour. Biol. Chem., 119, No. 2, p. 663, July, 1937.

The method is based on the turbidity of the unknown when treated with Nessler's solution as compared with a set of standards. The author gives the limitations of the method and the materials

that interfere. It does not differentiate acetone from other volatile ketones.

TWO TIME SAVING METHODS OF AFFIXING SECTIONS TO SLIDES: J. D. Schneidau, Jr., Trans. Amer. Mic. Soc., Vol. LVI, No. 2, p. 258, April, 1937.

A method for applying albumin solution is given. The author gives a formula that eliminates danger of excess albumin and also prevents complete drying of sections. The second method given uses no fixing solution.

DETERMINATION OF FERMENTABLE BLOOD SUGAR BY GASOMETRIC MEASUREMENT OF THE CARBON DIOXIDE FORMED BY THE ACTION OF YEAST: R. F. Holden, Jr., Jour. Biol. Chem., Vol. 119, p. 347, June, 1937.

Macro and micro fermentation methods using the Van Slyke apparatus are described. These are found to be applicable to as low as 5 cc. 1-10 blood filtrate and 0.12 cc. whole blood. The macro-method may also be used to determine urine sugar. The tests can be completed in 6 minutes.

THE DETERMINATION OF BILIRUBIN WITH THE PHOTO-ELECTRIC COLORIMETER: H. T. Malloy and K. A. Evelyn, Jour. Biol. Chem., Vol. 119, No. 2, p. 481, July, 1937.

A method for determining direct and indirect bilirubin in blood serum is given. The authors compare results obtained by this and other methods and conclude that the proposed method eliminates possible adsorption in protein precipitation and the interference of other yellow serum pigments.

A TITRIMETRIC METHOD FOR THE QUANTITATIVE ESTIMATION OF LEAD IN BIOLOGICAL MATERIALS: M. K. Horwitt and G. R. Cowgill, Jour. Biol. Chem., Vol. 119, No. 2, July, 1937.

A method is described for normal and pathological quantities of lead in 10 cc. samples of blood, 200 cc. samples of urine and small amounts of bone. The procedure may also be used on foodstuffs.

DIAGNOSIS OF ECTOPIC PREGNANCY: W. F. Fuller, M.D., Northwest Medicine, 36:4, April, 1937.

Repeated hematology, the urobilinogen test and sedimentation distance are stressed as diagnostic aids.

ROLE OF THE BIOPSY IN DIAGNOSIS: C. R. Jensen, M.D., Northwest Medicine, 36:4, April, 1937.

From the clinical standpoint, rather than the technical, the newer phases of biopsy work, grading of tumors and the limitations of pathologic diagnosis are discussed.

THE DIAGNOSTIC VALUE OF THE COMPLEMENT FIXATION TEST IN AMEBIASIS: Charles F. Craig, M.D., Revista de Parasitologia, Clinica y Laboratorio, 2:3, May-June, 1936.

The complement fixation reaction in amebiasis is specific and of practical value, especially in diagnosis of liver abscess, carriers and in the control of treatment. It should not replace a careful stool examination by one capable of differentiating the various intestinal amebae.

HEMOLYTIC BLOOD TRANSFUSION REACTION: Max W. and Robert W. Hemingway, M.D., Northwest Medicine, 36:2, Feb., 1937.

The authors report a fatal case of hemolytic transfusion reaction in which no incompatability could be shown by the usual methods before and after transfusion.

FACIAL CELLULITIS—INVESTIGATION OF ITS CAUSATIVE ORGANISM: Sachindra Kumar Sircar, Calcutta Medical Journal, 31:5, Nov., 1936.

The author thoroughly investigated nine cases by hemoculture, culture of the lesions, autopsy, bacterial examination of postmortem materials and animal inoculation and found a hemolytic staphylococcus in all cases. There seemed to be two types.

CARBOHYDRATE METABOLISM DURING EXPERIMENTAL HUMAN SALT DEFICIENCY: R. A. McCance, Biochem. Jour., p. 1276, August, 1937.

Glucose tolerance tests were given to the same subjects under normal conditions and during salt deficiency. The blood sugar curves were consistently higher during salt deficiency but not markedly so.

DISTRIBUTION OF IRON IN THE BLOOD: C. E. Jenkins and M. L. Thomson, Brit. Jour. Exp. Path., p. 175, June, 1937.

A colorimetric method was used to determine Fe in blood and the average non-Hb cellular Fe was found to be about 7 p.c. In anaemias there was much deviation from the normal. Hb cannot be accurately calculated on the basis of whole blood Fe.

THE NUTRITION OF STAPHYLOCOCCUS AUREUS: NICOTINIC ACID AND VITAMIN B: B. Knight, Biochemical Jour., Vol. 31, No. 5, p. 731, May, 1937.

The "growth factor" always necessary in medium of otherwise known chemical composition to permit growth of Staphylococcus aureus has been successfully provided by Nicotinic acid and Vitamin B., giving a medium containing only known chemical components, able to support growth of a typical strain.

A STUDY OF THE EFFECT OF OVERFEEDING ON THE PROTEIN METABOLISM OF MAN: D. P. Cuthbertson, A. McCutcheon and H. N. Munro. Biochemical Jour., Vol. 31, No. 4, p. 681, April, 1937.

A litre of milk added to a diet sufficient in nitrogen and adequate for body maintenance, caused an increase in weight and retention of nitrogen and sulfur but not of calcium.

AN IMPROVED PRECIPITATION REACTION VIEWING APPARATUS: L. C. Nickolls and J. C. Thomas, Brit. Jour. Exp. Path., p. 55, Feb., 1937.

The authors describe the construction of and explain the principles of an apparatus that serves to accentuate fine precipitates by their dispersion of parallel rays of light.

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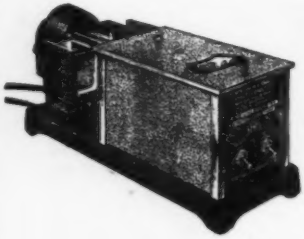

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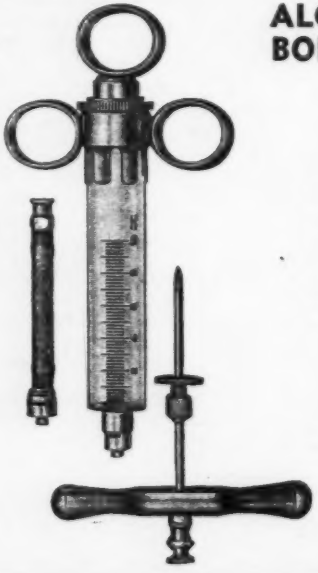

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See Journal A. M. A. 108: 803, March, 1937

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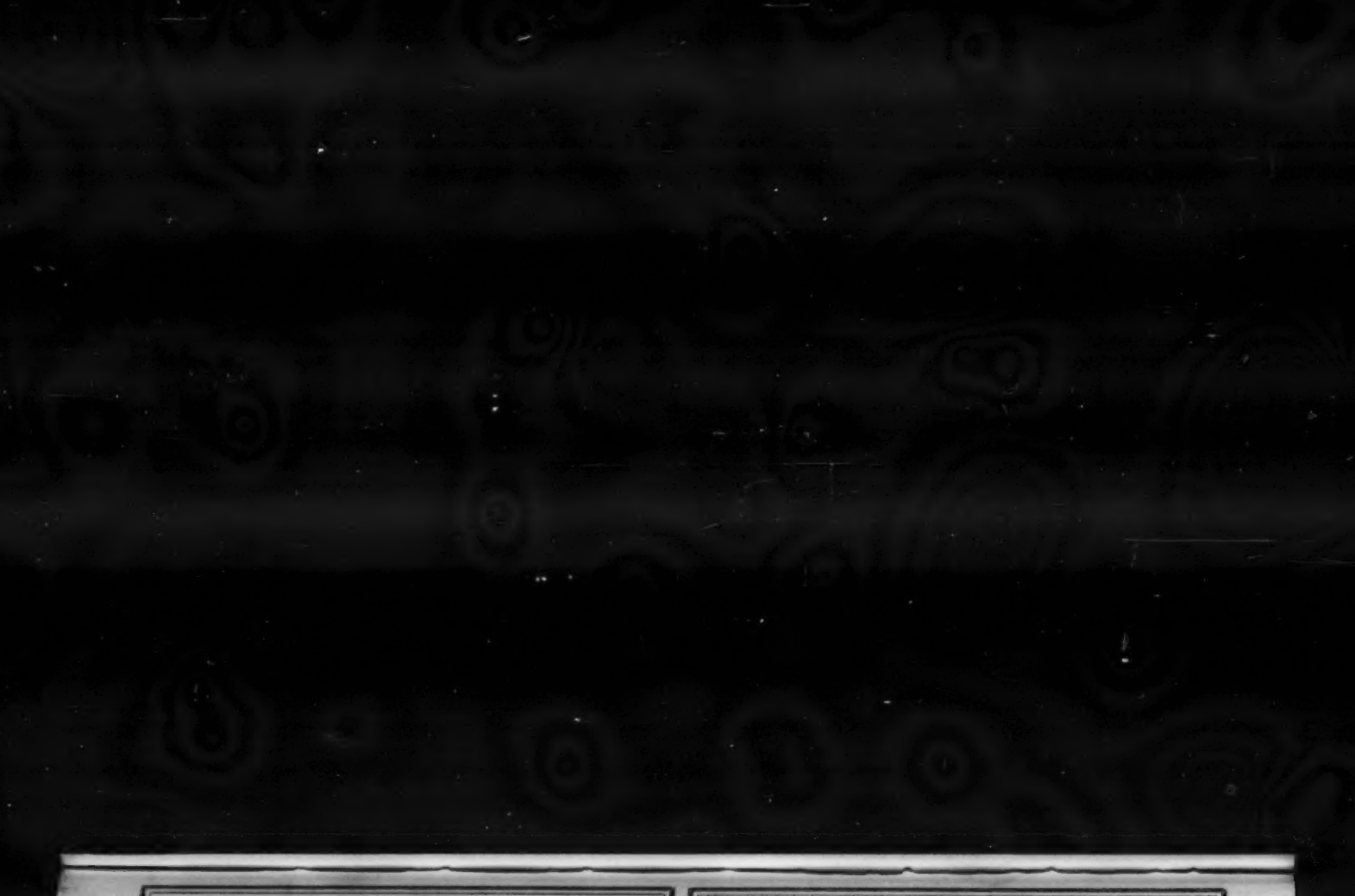
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